

# Diurnal Patterns in Phytoplankton Photosynthesis, Fremantle Harbour

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Phytoplankton are likely to be one of the main sources of autochthonous production in the Swan Canning estuary yet only limited published data are available on their photosynthetic dynamics. Here we describe the phytoplankton community composition at the mouth of the estuary and diurnal pattern of phytoplankton photosynthesis by applying chlorophyll fluorescence techniques. Diatoms were the dominant taxa (46–88% of the phytoplankton community) recorded throughout the sampling period although their dominance appeared to increase with the flood tide. A diurnal pattern in photosynthetic performance was apparent via chl-*a* fluorescence measurements. Night-time deactivation of Calvin-Benson cycle enzymes was evidenced by low  $rETR_{max}$  and light saturation point ( $E_k$ ; minimum value of  $207.5 \pm 2.3$  (se)  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) values. Observed daytime increases in  $E_k$  (maximum value of  $835.8 \pm 3.8$  (se)  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  at 4pm) with simultaneous reduction of  $\alpha$  suggests dynamic down-regulation of PSII electron transport during the day. Maximum quantum yield of PSII ( $F_v/F_m$ ) decreased from a value of 0.81 to minima of 0.51 during high-light periods of the day. Accounting for these dynamics is important in the design of future studies of phytoplankton productivity in the system. High recorded values of  $F_v/F_m$  and an unimpeded ability to photoregulate suggest the diatom-dominated marine phytoplankton community found in Perth's coastal waters at the time of this study was nutrient replete.

**KEYWORDS:** chlorophyll fluorescence, diurnal pattern, estuary, photoregulation, primary production, quantum yield

Abbreviations:  $\alpha$  = initial slope of the photosynthesis vs irradiance curve;  $E_k$  = saturation irradiance; ETR = Electron Transport Rate;  $F'$  = chl-fluorescence level under actinic light;  $F_m$  = saturation pulse-induced maximum chl-fluorescence level from a dark-adapted sample when all photosystem II reaction centres are closed and non-photochemical quenching is negligible;  $F_m'$  = maximum chl-fluorescence level from a light-adapted sample exposed to a saturation pulse, closing all all photosystem II reaction centres;  $F_o$  = minimum chl-fluorescence level from a dark-adapted sample when all photosystem II reaction centres are open;  $F_v$  = maximum variable fluorescence ( $F_m - F_o$ );  $F_v'$  = variable fluorescence under actinic light ( $F_m' - F_o'$ );  $F_v^q/F_m$  = maximum quantum yield of photochemistry at photosystem II;  $F_v^q/F_m'$  = effective quantum yield of photochemistry at photosystem II in the light; RCII = photosystem II reaction centre;  $rETR$  = relative electron transport rate;  $rETR_{max}$  = maximum relative electron transport rate.

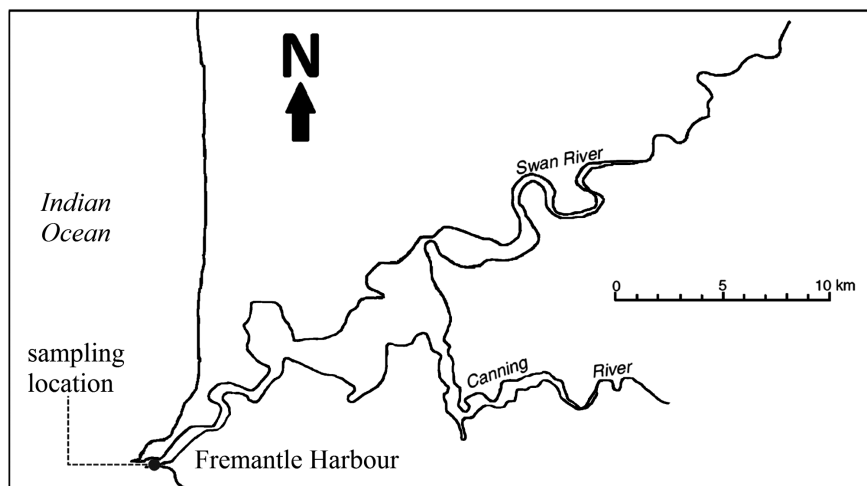
## INTRODUCTION

The city of Perth, Western Australia, surrounds the Swan-Canning Estuary. This microtidal, wave-dominated estuary is the second largest estuary in south-western Australia (Thompson 1998) and Fremantle Harbour is located at its mouth. Freshwater flow from tributaries is highly seasonal and a salt-wedge extends up the estuary from October to July/August (<http://www.wrc.wa.gov.au/srt/riverscience/>). The harbour is the transitional point between the oligotrophic coastal waters and the mesotrophic waters of the lower estuary.

Phytoplankton, microphytobenthos and seagrass are important sources of autochthonous production within the Swan-Canning Estuary (Hillman *et al.* 1995; Masini & Mccomb 2001) however there is a paucity of data on the photosynthetic dynamics of phytoplankton in the estuary and Perth coastal waters. While there have been some measurements of phytoplankton primary production (Thompson 1998), much of the literature has focused on the relationship between phytoplankton (chl-*a*, species composition) and environmental factors such as salinity, river flow, temperature and dissolved nutrients (Thompson 1998; Thompson 2001; Twomey & John 2001). Active chlorophyll fluorometry allows rapid estimation of photosynthetic parameters (e.g. quantum yield, electron transport rate) however the number of studies applying this technology to investigate diurnal patterns in phytoplankton photosynthesis *in situ* and with high sampling frequency is limited (Dijkman & Kromkamp 2006; Kurzbaum *et al.* 2010; Mackey *et al.* 2008; Verspecht 2007; Zhang *et al.* 2008).

Previous studies suggest that daily endogenous rhythms in the maximum rate of photosynthesis ( $P_{max}$ ) and  $\alpha$  occur in all major taxonomic groups of phytoplankton and that these rhythms are independent of changes in chl-*a* content (Harding *et al.* 1982; Boyd *et al.* 1997; Behrenfeld *et al.* 2004). Such rhythms should be taken into account when designing experiments investigating phytoplankton photosynthesis and interpreting results.

This study aims to describe the phytoplankton community present in Perth's coastal waters and investigate any diurnal pattern of photosynthesis by applying chlorophyll fluorescence techniques.



**Figure 1.** The study site on the seaward edge of Fremantle Harbour, the mouth of the Swan-Canning Estuary (modified from Hamilton *et al.* (2006)).

## METHODS

### Study site and environment

The study site was located at the mouth of Fremantle Harbour, with sample collection from a small motorised boat between the Maritime Museum of Western Australia and the OceanFarm jetty (Figure 1; GPS coordinates: 32°03'18.90" S 115°44'14.93" E). All sample collection and on-site measurements were performed on 7<sup>th</sup> December, 2005. Data on solar irradiance ( $W m^{-2}$ ) incident at sea level were taken from Murdoch University meteorological station data (located 9.4 km from Fremantle Harbour; <http://www.met.murdoch.edu.au/downloads.htm>). These data were converted to photosynthetically available radiation (400–700 nm) in units of  $\mu mol\ quanta\ m^{-2}\ s^{-1}$  following the method of Thompson (1998). Tidal information was collected from the Coastal Data Centre (<http://www.dpi.wa.gov.au/imarine/coastaldata/1895.asp>) and on-site observations, while water surface temperature was recorded during each sampling occasion with a mercury thermometer.

### Phytoplankton composition and chlorophyll

Samples for phytoplankton and chlorophyll were collected at 03:00 and every second hour until 19:00. A final sample was collected at 22:00 to yield a total of 10 samples for each analysis.

For phytoplankton identification and enumeration, at each sampling period a 1 L of near-surface (~0.5 m) seawater was collected from the end of the OceanFarm jetty into a thrice-rinsed Nalgene® bottle and 1 mL of Lugols iodine solution added. Analysis was performed using a Sedgewick-Rafter chamber at 200× magnification under a light microscope. Samples had previously been allowed to settle (>7 days) and concentrated 10–15 fold. Cell density calculation took concentration and dilution (due to addition of Lugols) into account. The most dominant phytoplankton were identified to species level using Tomas (1997), however in most instances cells were categorised into major taxonomic groupings only.

A further ~5 L near-surface (~0.5 m) grab sample was collected for chlorophyll-*a* determination. Samples were kept cool and in dim light until they could be returned to the university laboratory (no longer than

2 h) for filtration through Whatman GF/F (45 mm) at a pressure of  $40 \pm 10$  kPa. Filters were patted dry, wrapped in aluminium foil and frozen at  $-80^{\circ}C$  for later extraction. Extraction was performed with ice-cold 90% (v/v) acetone by manual grinding using a glass mortar and pestle (modified from Strickland and Parsons (1972)). The homogenate was set in ice and away from light for 30 min and then clarified by centrifugation at 2100 rpm (1000 g) for 15 min in a Beckman GPR model centrifuge at  $4^{\circ}C$ . The centrifuge vials were then returned to ice and the supernatant used to determine chl-*a* content spectrophotometrically using the equations of Jeffrey and Humphrey (1975) for chromophyte algae containing chlorophylls *a*, *c*<sub>1</sub> and *c*<sub>2</sub>. A Beckman DU-50 (UV-VIS) series spectrophotometer was used for all spectrophotometric measurements.

### Chlorophyll fluorescence

Samples for chl-fluorescence measurements were collected from a small motorised boat using a 25  $\mu m$  mesh-size phytoplankton net towed just below the surface until concentration of phytoplankton was sufficient to yield a satisfactory signal to noise ratio in the fluorometer. This generally took about 20–25 minutes. Upon return to the shore the suspension in the phytoplankton net cod-end was passed through a 180  $\mu m$  mesh to remove zooplankton prior to sample measurements.

All fluorescence measurements were performed with a Water-PAM fluorometer (Walz, Germany) consisting of a Water-ED emitter-detector unit and a PAM-Control box. Data collection was via the WinControl (v2.08) software provided with the fluorometer. The fluorescence terminology used here follows that of Baker and Oxborough (2004). Samples were collected for fluorescence analysis on an hourly basis from 03:00 until 22:00 and were kept in dim light (shade) until measured.

Rapid light curves (RLCs) were performed to measure relative electron transport rate (rETR). Light levels were chosen, based on preliminary measurements (data not shown), to achieve a balance between maintaining as many points in the light-limited region of the curve while allowing an asymptote or down-turn to be evident. Gain levels were adjusted to maintain a dark fluorescence signal (i.e. measuring light only) of 200–300 units. Light

width at each irradiance was 5 s and saturation pulse duration was 0.8 s. For each sampling period ten replicate RLCs were performed on light adapted samples (kept in shaded conditions during collection procedure and during any delay before measurement). Ten replicate dark adapted  $F_v/F_m$  measurements were also collected at each sampling period. Dark-adaptation was for 15 minutes and samples were exposed to 5 s of far-red light immediately prior to applying the saturation pulse. rETR was calculated as (Cosgrove & Borowitzka 2010). Data were imported into Matlab® (Mathworks) and the model of Eilers and Peeters (1988) was fitted to the combined data (10 replicates) for each treatment to generate photosynthesis-irradiance (P-E) curve parameters. The error of the parameter estimates was derived from the covariance matrix of the fitted model and expressed as standard error.

## RESULTS

### Site conditions

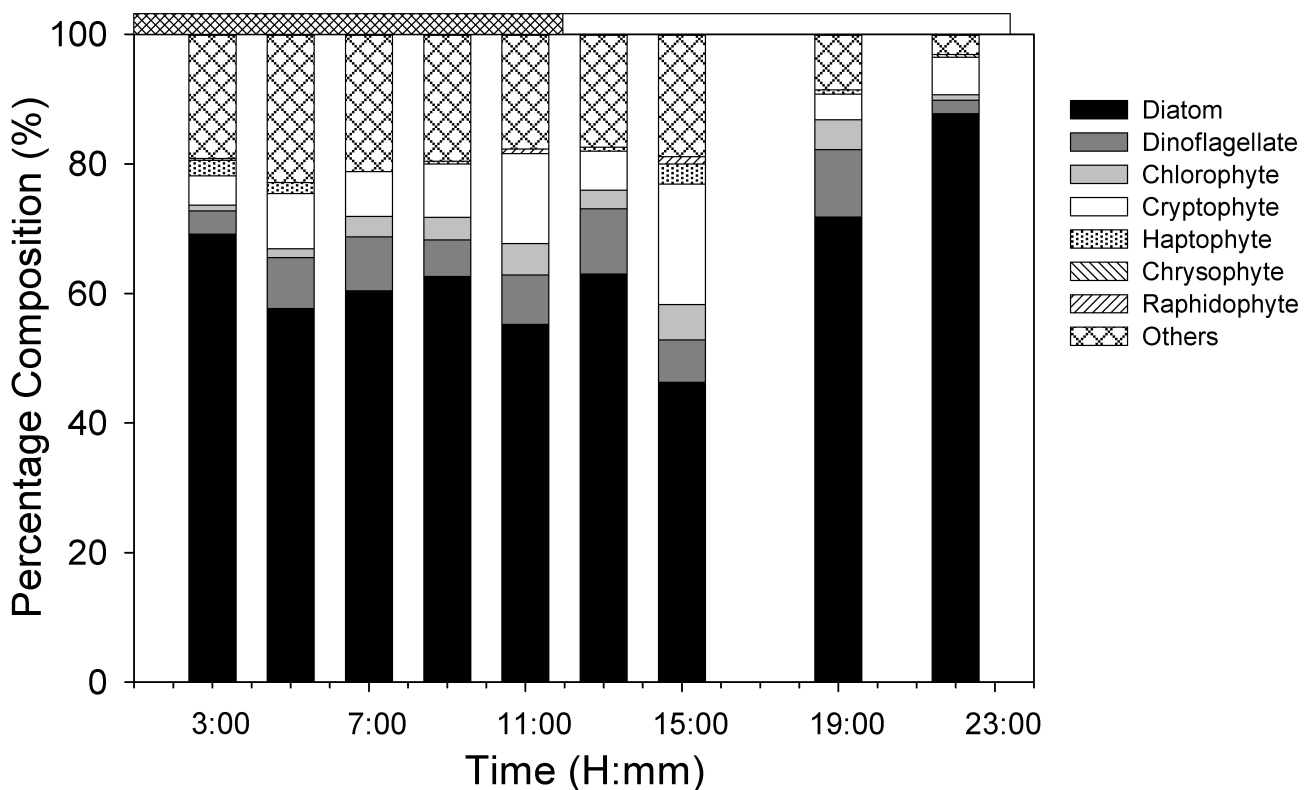
Water temperature was 18°C during the early morning and late evening and 20°C throughout the day from 10:00 to 18:00. Lower temperatures coincided with ebb tide while higher temperatures occurred during flood tide.

### Species composition, cell density and biomass (chl-a)

Diatoms (Bacillariophyceae) were found to be the

dominant taxon throughout the sampling period. Diatoms were most dominant in the evening, representing a maximum of 88% of the phytoplankton community at 22:00 and a minimum of 46% at 15:00 (Figure 2). The highest percentage of diatoms occurred close to the end of the flood tide. The large species *Chaetoceros lorenzianus* and *C. curvisetus* were dominant at 15:00 while the small species (unidentified and referred to as *Chaetoceros* sp1.) was dominant in the evening (Table 1). The most commonly abundant species during the sample period were: *Chaetoceros* sp1., *Pseudonitzschia seriata*, unidentified nanoflagellates and *C. curvisetus*. Due to the predominance of diatoms throughout the entire sampling period it was assumed that changes in species composition had negligible impact on chl-fluorescence measurements.

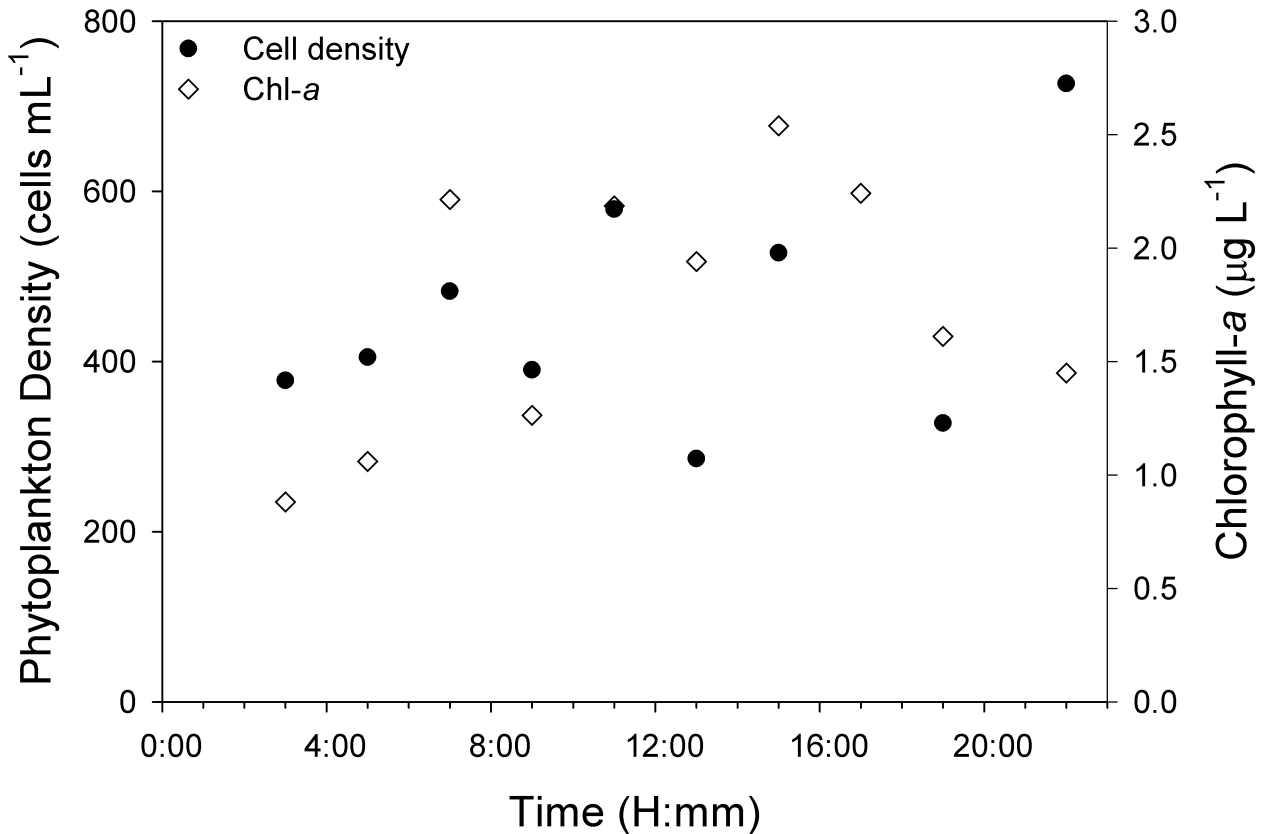
The chl-*a* concentration of the marine waters at the mouth of Fremantle Harbour increased throughout the day, reaching a peak of 2.54  $\mu\text{g L}^{-1}$  at 15:00 before decreased in the evening towards the end of flood tide. Average chl-*a* concentration across the entire sampling period was  $1.73 \pm 0.18(\text{se}) \mu\text{g L}^{-1}$  (n=9). While phytoplankton cell density was generally positively related with chl-*a* concentration, there were some periods where dominance of large (i.e. 13:00) or small (i.e. 22:00) species yielded relatively high or low chl-*a* concentrations, respectively ( $r^2$  shifts from 0.066 (n=9) to 0.592 with these two sampling times removed; data not shown).



**Figure 2.** Percentage composition of phytoplankton taxa in Fremantle Harbour over the duration of the study period. Ebb tide is indicated by the thatched bar at the top of the graph while flood tide is indicated with an open bar.

**Table 1.** Fremantle Harbour phytoplankton community composition and relative abundance at ~0.5 m depth. Grab samples were collected every second hour throughout the day. (+) present, (++) present in moderate relative abundance, (+++) present at a high abundance relative to other species.

	3 AM	5 AM	7 AM	9 AM	11 AM	1 PM	3 PM	5 PM	7 PM	10 PM
<b>Diatoms</b>										
<i>Bacillaria cf. paradoxa</i>			+							
<i>Cerataulina</i> sp.	+						+		+	+
<i>Chaetoceros curvoisetus</i>	+			+++	+++	+++	+++		+	+
<i>Chaetoceros cf. lorenzianus</i> var. <i>forceps</i>		+					+			
<i>Chaetoceros cf. laciniatus</i>			+		++	++	+++		++	
<i>Chaetoceros decipiens</i>				+	++	+			+	+
<i>Chaetoceros tenuissimus</i>						+				
<i>Chaetoceros</i> sp1.	+++	+++	+++	+++	++	+++	+++		+++	+++
<i>Cylindrotheca closterium</i>	+	+	+	+	+	+	+		++	+
<i>Cyclotella meneghiniana</i>				+	+	+	+			+
<i>Diploneis</i> sp.			+	+						
<i>Entomoneis</i> sp.	+	+	+	+		+				
<i>Guinardia</i> sp.										
cf. <i>Gyrosigma</i> sp.						+	+			
<i>Licmophora</i> sp.					+				+	
<i>Navicula</i> spp.	+	+	+	+	+	+	+		+	+
<i>Nitzschia</i> spp.	+	+					+		+	
<i>Pseudonitzschia seriata</i>	+++	+++	+++	+++	++	++	++		++	+++
<i>Pseudonitzschia pseudodelicatisima</i>	+	+							+	+
cf. <i>Roicosigma</i> sp.						+				
<i>Skeletonema costatum</i>	++	+	+++	+		+++			+	+
cf. <i>Striatella</i> sp.									+	
<i>Thalassionema</i> sp.	+	+	+	+	++	++	+		+	+
<i>Thalassiosira</i> spp.			+						+	
<b>Dinoflagellates</b>										
<i>Amphidinium</i> sp.			+			+	+		+	
<i>Ceratium furca</i>	+		+			+	+		+	+
<i>Dinophysis acuminata</i>					+					
<i>Diplopsalis</i> sp.						+				
<i>Gonyaulax</i> sp.									+	
<i>Gymnodinium</i> spp.		+	+	+	+	+	+			+
<i>Gyrodinium</i> spp.	+	+	+	+	+	+	+			
<i>Katodinium glaucum</i>	+				+					
<i>Katodinium rotundata</i>		+	+		+	+	+		+	+
<i>Polykrikos</i> sp.									+	
<i>Prorocentrum minimum</i>		+	+		+	+	+		+	+
<i>Prorocentrum micans</i>			+	+	+	+	+			
<i>Prorocentrum triestinum</i>									+	+
<i>Protoperdinium</i> spp.	+		+		+	+			+	+
<i>Scrippsiella cf. trochoidea</i>	+	+					+		+	
<b>Cryptophytes</b>										
cf. <i>Plagioselmis</i> spp.		+	+	+	+	+	+		+	+
cf. <i>Rhodomonas</i> sp.					+					
cf. <i>Teleaulax</i> sp.	+		+			+	++		+	
<b>Prasinophytes</b>										
<i>Pyramimonas</i> spp.	+	+		+	+	+	+		+	+
<i>Tetraselmis</i> sp.		+	+	+	+		+		+	
<b>Euglenoids</b>										
<i>Eutreptiella</i> sp.		+	+		+					
<b>Chrysophytes</b>										
<i>Apedinella</i> sp.	+	+								
<i>Pseudopedinella</i> sp.	+	+		+		+	+		+	+
<b>Raphidophytes</b>										
<i>Heterosigma akashiwo</i>	+				+		+			
<b>Silicoflagellates</b>										
<i>Dictyocha fibula</i>		+	+							+
<b>Others</b>										
<i>Ebria tripartita</i>							+			
<i>Mesodinium rubrum</i>	+	+	+			+	+		+	+
Nanoflagellates	++	++	++	+++	++	++	+++		+	+



**Figure 3.** Phytoplankton cell density and chl-*a* concentration in Fremantle Harbour over the duration of the study period. Inconsistencies at 1300 hrs and 2200 hrs relate to changes in species composition.

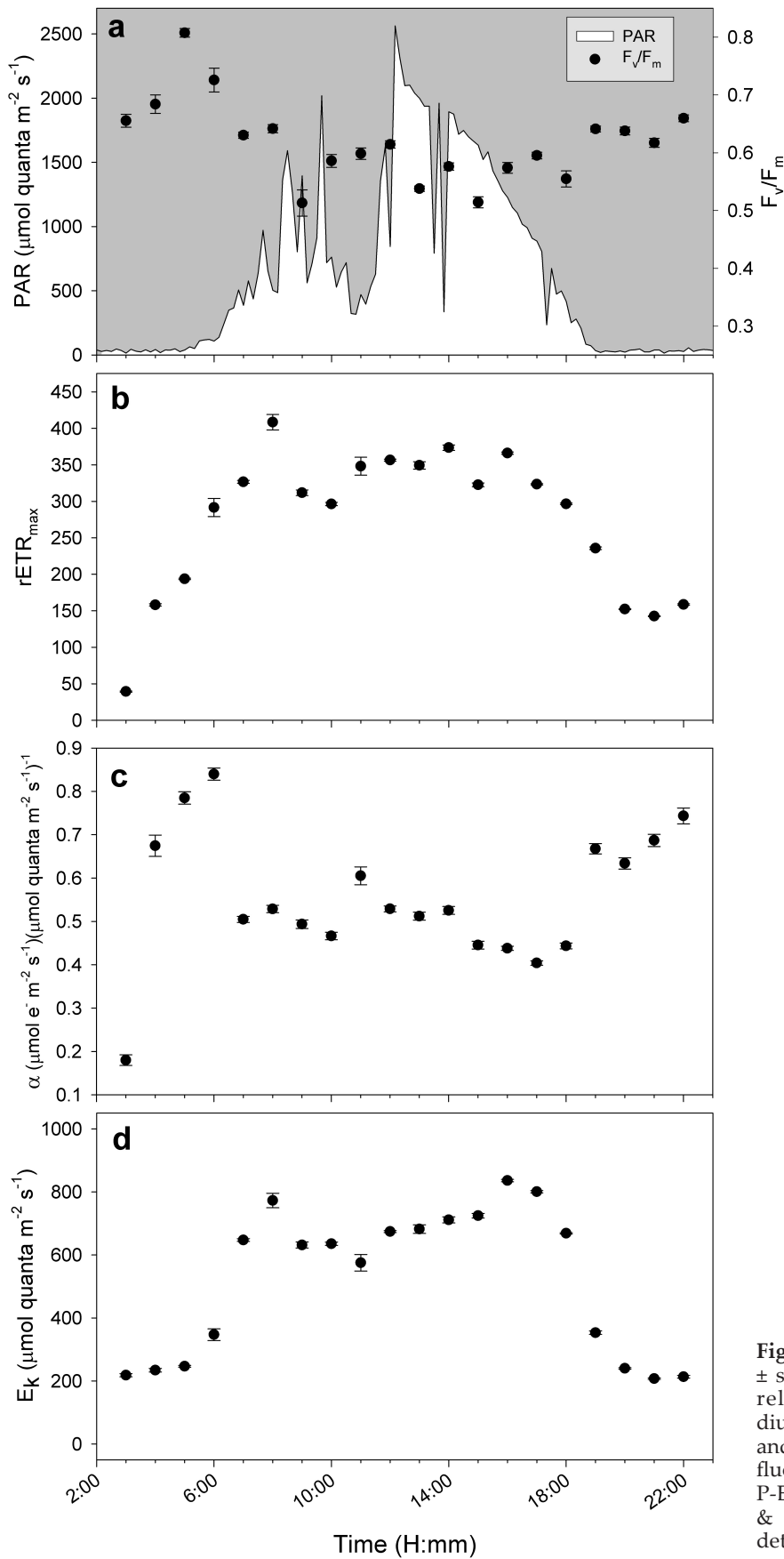
#### Diurnal variability in photosynthesis

Analysis of  $F_v/F_m$  data revealed a typical diurnal cycle pattern (Figure 4a).  $F_v/F_m$  was quite high ( $\sim 0.65$ ) at 03:00 and 04:00, however immediately prior to dawn  $F_v/F_m$  increased to a value of  $0.81 \pm 0.01$  (error = se,  $n = 10$ ). This value was very close to theoretically maximal values of 0.83 (Magnusson 1997). A rapid decrease in  $F_v/F_m$  occurred over the early daylight hours and, throughout most of the day, values between 0.57 and 0.60 were observed.  $F_v/F_m$  dropped below this to values of  $0.51 \pm 0.02$  at 09:00,  $0.54 \pm 0.01$  at 13:00 and  $0.51 \pm 0.01$  at 15:00. Each of these minima were associated with a period of high incident irradiance during the sample collection period (Figure 4a).

The maximum photosynthetic rates as measured by chl-fluorescence ( $rETR_{max}$ ) indicate that photosynthetic processes were substantially down-regulated during the night (Figure 4b). Re-induction of these processes was rapid and possibly even predictive, since photosynthetic capability, as indicated by  $rETR_{max}$ , began to increase prior to dawn. This development of photosynthetic capacity was also reflected by the sudden and large increase in  $\alpha$  (Figure 4c).  $rETR_{max}$  reached a peak at 08:00 ( $408 \pm 10.6$  relative units, error = se,  $n = 10$ ) before declining again as the first periods of high irradiance ( $852 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  at 08:30; Figure 4a) resulted in downregulation of photosynthesis and potentially some photoinhibition. Downregulation was indicated by the earlier and proportionately greater

drop in  $\alpha$  compared to  $rETR_{max}$  leading to higher values for  $E_k$  (Figure 4d).

The depression of  $F_v/F_m$  at 09:00 and a concomitant decrease in  $rETR_{max}$  and  $E_k$  after a gap in the clouds resulted in the first period of high irradiance for the day (Figure 4), suggested photoinhibition due to damage of the photosynthetic apparatus may have occurred. A substantial recovery of  $F_v/F_m$  from  $0.51 \pm 0.02$  (error = se) at 09:00 to  $0.59 \pm 0.01$  in the following hour suggests that down-regulation rather than photoinhibition was responsible for the downturn in photosynthesis; however a continuing slow recovery of  $F_v/F_m$  over the next 2 hours of cloud cover (minima for this period was  $318 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  at 10:50) indicates that some photodamage did occur. Depression of  $F_v/F_m$  was observed again at 13:00 and 15:00 after extended periods of high light, with the 15:00 depression being accompanied by decreases in  $rETR_{max}$  and a despite slightly lower incident irradiances (Figure 4). By dusk  $rETR_{max}$  began to decrease substantially despite large increases in photosynthetic efficiency (as indicated by  $\alpha$ ). This suggests substantial rate limitation by the Calvin-Benson cycle as its associated light activated enzymes begin to 'switch off'. The diurnal pattern of a closely reflected  $F_v/F_m$  changes, given that the initial slope is the product of the chl-specific absorption coefficient and maximum photosynthetic efficiency, this suggests that the light absorption characteristics remained relatively stable throughout the day.



**Figure 4.** (a) Phytoplankton  $F_v/F_m$  (mean  $\pm$  se,  $n = 10$ ) over the course of the day relative to incident irradiance. The diurnal changes in (b)  $rETR_{\text{max}}$ , (c) alpha and (d)  $E_k$  parameters measured by chl-*a* fluorescence as determined by modelling P-E curve data with the model of Eilers & Peeters (1988) (error bars = se as determined by the model).

## DISCUSSION

The phytoplankton community in the coastal marine waters sampled at the mouth of the Swan-Canning Estuary (this study) was found to be dominated by members of the Bacillariophyceae (diatoms) (Figure 2, Table 1). Dominance of diatoms in both near-shore coastal waters (Thompson & Waite 2003) and in the lower reaches of the Swan-Canning Estuary (Thompson 1998) has been described previously. The persistent dominance of diatoms throughout the study was fortunate, as shifts in taxonomic composition between species with different pigments (light harvesting and accessory) and/or thylakoid arrangements can influence the signature obtained by PAM fluorometers (Büchel & Wilhelm 1993). Thus, the observed taxonomic stability gave some confidence to the assumption that changes in fluorescence signal represented changes in physiological state of the phytoplankton community rather than taxonomic shifts.

Chl-*a* concentrations ranged between 0.88  $\mu\text{g L}^{-1}$  at 03:00 to 2.54  $\mu\text{g L}^{-1}$  at 15:00 (Figure 3). Peaks in chl-*a* concentration above  $\sim 1.6 \mu\text{g L}^{-1}$  were dependent on the presence of large, chlorophyll-rich diatom species such as *Chaetoceros curvicaetus*, *Chaetoceros lacinosus* and *Skeletonema costatum*. The chl-*a* concentrations here were similar to those recorded at Blackwall Reach, about 6 km upstream, by Thompson (1998), although cell densities were substantially lower. Despite the Water-PAM's reported lower-limit of chl-*a* detection being 0.1  $\mu\text{g L}^{-1}$ , the fluorescence signal was found to be unstable and sample concentration was required in order to achieve a stable and reliable fluorescence trace over the course of fluorescence measurements. Concentration of samples via the use of a plankton net may shock phytoplankton cells and can result in substantially lowered quantum yield measurements (Peter Ralph, pers. comm.). However, given that  $F_v/F_m$  values recorded after the concentration procedure were as high as  $0.807 \pm 0.007$  (error = se,  $n = 10$ ; Figure 4a), any negative impact on phytoplankton physiology was considered negligible.

Endogenous diel oscillations in the P-E response of marine phytoplankton have been widely reported (Boyd *et al.* 1997; Harding *et al.* 1981; Harding *et al.* 1982; Harris 1980) and diatoms appear to display greater diel periodicity than most other taxa (Behrenfeld *et al.* 2004; Harding *et al.* 1981). Studies have found maximum photosynthetic rate ( $P_{\text{max}}$ ) to peak both in the morning (Marra *et al.* 1985) and afternoon (Harding *et al.* 1982). Timing differences in the pattern of diel changes have previously been explained by changes in latitudinal photoperiod and variations in nutrient assimilation and utilisation between phytoplankton size fractions (Harding *et al.* 1982). More generally, diel rhythms in  $P_{\text{max}}$  and  $\alpha$  are commonly understood to result from oscillations between metabolic pathways which are influenced by light periodicity and exogenous nutrient supply (Harding *et al.* 1982; Behrenfeld *et al.* 2004). Nutrients accumulated during dark periods may help to yield a high  $P_{\text{max}}$  the following morning (Erga & Skjoldal 1990). Henley (1993) states that maximum photosynthetic rate usually peaks in the morning or close to midday and that this pattern may be endogenous, rather than related to chl-content, photoinhibition, nutrition or feedback inhibition by accumulated photosynthate. In this study,

the observed pattern in  $r\text{ETR}_{\text{max}}$  appear to be a response to the changing light environment; with increasing values in the morning as key Calvin-Benson cycle enzyme such as Ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBisCo) become activated at dawn ("light activation") (Buchanan 1980) and down-regulation or inhibition during the middle part of the day as RCII's become closed or damaged (Figure 4a, b). However, the significant increase in  $r\text{ETR}_{\text{max}}$  prior to dawn suggests that, to some extent, an endogenous rhythm was also present.

The PAM chl-fluorescence parameters  $F_v/F_m$ ,  $r\text{ETR}_{\text{max}}$  and  $F_0$  indicate possible photoinhibition of phytoplankton photosynthesis at 09:00, 13:00 and 15:00 (Figure 4). Measurements at 09:00 were taken shortly after a break in cloud cover resulted in surface irradiances of  $\sim 1500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  and  $F_v/F_m$  was found to be depressed ( $0.51 \pm 0.023$  compared to  $0.64 \pm 0.007$  at 08:00). This drop in  $F_v/F_m$  was accompanied by a lower recorded  $r\text{ETR}_{\text{max}}$  while a had undergone a sudden decrease at 07:00 and subsequently remained relatively stable. A common measure of photosynthetic efficiency and physiological stress, the chl-fluorescence parameter  $F_v/F_m$  is extensively used to describe diurnal patterns in photosynthesis. Diurnal variability in  $F_v/F_m$  has been shown to be closely related to incident irradiance and midday depression of  $F_v/F_m$  is frequently observed when irradiance is high (ie. no or limited cloud cover) (e.g. Bergmann *et al.* 2002). However, it should not be assumed that  $F_v/F_m$  maxima and minima always occur during the evening and around midday respectively. Behrenfeld and Kolber (1999) describe measurements taken in the South Pacific Ocean (prokaryote-dominated phytoplankton community in iron limited conditions) that exhibit nocturnal  $F_v/F_m$  minima. Reduction of  $F_v/F_m$  can result from a number of physiological processes including numerous pathways for down-regulation of photosynthesis and closure of photosystem II reaction centres (RCII's) due to photoinhibition associated with damage to the D1 protein within the core of photosystem II (Adams III *et al.* 2006; Cartaxana *et al.* 2013; Edelman & Mattoo 2006). Changes in chl-fluorescence can be used to make inferences about the photophysiological state of phytoplankton cells in the sample. A disproportionate reduction of  $F_0$  compared to  $F_m$  may represent enhanced xanthophyll cycle activity (photoprotection) or enhanced NADPH-reductase activity leading to state transition and photosystem II being in state-2 in the dark (Demmig & Björkman 1987; Magnusson 1997; Fracheboud 2001); while a decline in  $F_m$  and increase in  $F_0$  can indicate photoinhibition and closure of damaged reaction centres (Campbell *et al.* 2003; Franklin *et al.* 1992). The sampling methods undertaken did not allow for comparison of  $F_0$  and  $F_m$  between sampling periods and thus achieving a more quantitative assessment of the proportion of photoinhibition compared to photoregulatory down-regulation was not possible. The low signal to noise ratio of the conducted PAM chl-fluorescence measurements meant that quenching parameters were considered inconclusive (data not shown).

The highest irradiances for the day were recorded between midday and 13:00 as cloud cover that had persisted for most of the morning dissipated (Figure 4a). The  $F_v/F_m$  of the phytoplankton community

decreased from  $0.61 \pm 0.007$  at midday to  $0.54 \pm 0.005$  at 13:00 (Figure 4a), however on this occasion there was no concomitant decrease in  $rETR_{max}$  (Figure 4b). Data from Olaizola *et al.* (1994) suggest that changes in diadinoxanthin cycle pigment pool size could occur between hourly sampling periods. An increase in dissipative diatoxanthin content relative to chl-*a* would result in a decrease in both  $F_v$  and  $F_o$  (Lavaud *et al.* 2004), however  $F_v/F_m$  would also decrease as the drop in  $F_m$  is proportionately greater. It has been proposed that epoxidase activity is inhibited after exposure to high light (Olaizola *et al.* 1994). Thus the low  $F_v/F_m$  recorded during this study at 13:00 may be the result of diatoxanthin remaining de-epoxidized in the dark and increased levels of non-photochemical quenching rather than significant photodamage to RCII.

Similar to the pattern seen at 09:00, at 15:00 the drop in  $F_v/F_m$  was associated with a concomitant reduction of  $rETR_{max}$  indicating that some photoinhibition may have occurred (Figure 4a, b). However, substantial recovery of  $F_v/F_m$  was observed within the hour after each decline, indicating that that this was more likely to be an expression of dynamic regulatory mechanisms rather than photoinhibition (recovery from photoinhibition occurs on a time scale of hours to days).

Evidence of active quenching mechanisms was present in the pattern of  $E_k$ . Boyd *et al.* (1997) interpreted a significant decrease in  $E_k$  towards evening as relaxation of non-photochemical quenching. In this study, the substantially higher  $E_k$  values during the daylight hours compared to before dawn and after dusk (Figure 4d) resulted from a significant decline in  $\alpha$  (Figure 4c) with little or no reduction in  $rETR_{max}$  (Figure 4b) and can be construed as an increase in non-photochemical quenching. Also, the diurnal pattern of the P-E curve parameters tends to represent a photoacclimation response type A, as described by Richardson *et al.* (1983), where the activity of accessory pigments is thought to play a major role.

Diatoms, via the diadinoxanthin cycle, are capable of efficient downregulation of photosynthesis via non-radiative dissipation of excess energy (Cartaxana *et al.* 2013; Lavaud *et al.* 2002). The data presented here suggest that the diatom-dominated marine phytoplankton community found in Perth's coastal waters at the time of this study was capable of avoiding photoinhibitory damage by employing this tactic.

Along with the recorded high  $F_v/F_m$  values, the ability of the phytoplankton community to effectively regulate photosynthesis and avoid photodamage suggests that they were not nutrient limited. However, the nutrient concentrations of Perth's coastal waters have been described as "in the lower part of the range reported for temperate coastal waters elsewhere" (Johannes *et al.* 1994) with low N:P ratios resulting in a likelihood of N-limitation (Thompson & Hosja 1996). It is possible that phytoplankton at the mouth of the Swan-Canning Estuary at the time of this study were efficiently recycling nutrients from within the plankton community, or turbulence of the water column due water movement through the mouth of estuary provided sufficient nutrients via suspension of benthic solids however further studies would be needed to quantitatively

determine phytoplankton nutrient status and the source of these nutrients.

## CONCLUSION

The marine phytoplankton community at the mouth of the Swan-Canning estuary exhibited a typical diurnal pattern of photosynthesis, with maximum rates of photosynthesis occurring during the daylight hours as photosynthetic processes became rapidly induced. Studies assessing the productivity of microalgae in the system would need to take such diurnal variations into account.

High  $F_v/F_m$  values, reaching close to the theoretical maximum just prior to dawn, suggested that phytoplankton were physiologically competent and not nutrient-limited. The fast recovery of  $F_v/F_m$  after periods of high light while maintaining near maximal rates of photosynthesis ( $rETR_{max}$ ) supports this finding and highlights the photoprotective ability of diatoms, which were the dominant taxon.

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